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The Protective Role of Vitamin C against Histological Changes and Some Biochemical Indices in Liver of Rats Chronically Exposed to Diazinon

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ABSTRACT

Background: Diazinon is one of the most toxic organophosphrous pesticides. It is used widely in agriculture and affects the general health and the economy of the human. Toxic effect of Diazinon is due to inhibition of acetylcholine esterase, an enzyme needed for proper nervous system function. This study was designed to investigate the effects of Diazinon on the liver tissue and the expected protective role of vitamin C. **Aim of this study:** is to evaluate effect of Diazinon and vitamin C on liver of albino rats. **Results:** data showed a significant increase in liver enzymes AST, ALT in addition to GGT and decreased total proteins and albumin as well as different histological changes caused by the pesticide Diazinon. Using vitamin C caused amelioration in liver structure and function tests, although, all these tests did not return to the normal level. **Conclusion:** the present study proved that Diazinon has adverse effects on some biochemical parameters and liver functions leading to histological impairment and these effects increase with the increased time of exposure. Meanwhile, supplementation with vitamin C could ameliorate the adverse effects of Diazinon.

Keywords: Diazinon, Vitamin C, Serum enzymes, Liver histology, Rats

INTRODUCTION

Diazinon is an organophosphate insecticides and acaricide developed in the early 1950s.**Handy *et al*.**[1]reported that Diazinon caused toxic effects on blood cells, spleen, thymus and lymph nodes of rats**.**

Diazinon is a non-systemic insecticide used in agriculture to control soil and foliage insects and pests on a variety of fruits, vegetables and field crops. Diazinon is also used on non-lactating cattle in an insecticidal ear tag. Prior to the cancellation of all residential uses by 2004, Diazinon was used outdoors on lawns and gardens, indoors for fly control and in pet collars designed to control fleas and ticks[2].

Diazinon caused changes in liver enzymes and biochemical indices and swelling of mitochondria in hepatocytes and it also has been linked to the development of serious histopathological lesions in the kidneys and the brain[3-5].

Some authors demonstrated that vitamins such as C and E can be used to counteract pesticide toxicity in the experimental animals[6-8]. Vitamin C is an important water-soluble chain-breaking antioxidant and enzyme cofactor [9]**.**

MATERIALS AND METHODS

Experimental design and used animals:

Thirty six adult male albino rats (*Rattus norvegicus*) about 90 ±10gm weight were obtained from the Animal Breeding House of the Research Institute of Ophthalmology, Giza, Egypt. Animals were housed in metal cages for one week, as an acclimatization period, under the laboratory conditions. The rats were fed a commercial balanced ration and allowed free to excess of water. Abnormally noticed animals were eliminated. Thereafter, the animals were categorized into six groups in separated cages (40×60×30 cm), each group contained 6 individuals.

- Experimental Groups:

1- The Control group (C1): without any treatment or additives for the ration or water for (5weeks).

2- Diazinon group (D): received Diazinon (64 mg/kg b. w. Half of LD_{50}) twice weekly for the whole period of the experiment (5weeks).

3- Diazinon + Vitamin C group (D+C): rats given Diazinon (64 mg/kg b. w. Half of LD50 twice weekly) and supplemented with Vitamin C, daily, (5 mg/kg) for all the experiment period (5 weeks).

4- The Control group (C2): without any treatment or additives for the ration or water for (10 weeks).

5- Diazinon group (D): received Diazinon (64 mg/kg b. w. Half of LD50) twice weekly for the whole period of the experiment (10 weeks).

6- Diazinon + Vitamin C group (D+C): rats given Diazinon (64 mg/kg b. w. Half of LD50

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twice weekly) and supplemented with Vitamin C (5 mg/kg) for all the experiment period (10 weeks).

The Practical Laboratory Part:

Rats were taken oral administration of antioxidant (Vitamin C) daily followed by oral administration of the insecticide (Diazinon) two times a week. Normal ration and water were *ad libitum*. The experiment continues for 5 and 10 weeks.

At the end of five or ten weeks of the experiment, rats were sacrificed, samples from liver were collected and serum was obtained at once, blood samples were centrifuged at 3000 rpm for 10 min. Non hemolized serum was then aspirated into clean dry Ependorph and stored at-20 C till used for the biochemical analysis.

Serum Liver Function tests:

The serum Aspartate Transaminase (AST); alanine transaminase (ALT); serum γ-glutmate transaminase (GGT) and serum alkaline phosphatase (ALP) were estimated by using Bio-Merieux kits. Also, serum total bilirubin (T. Bili); serum total protein (T. P.) and albumin (Alb.) were estimated by using Bio-Merieux kits.

Statistical Analysis:

The Statistical analysis of the obtained data was done as t-test according to Quattro Pro for windows program version (2) Microsoft Windows version (7). The obtained data were assessed by calculation of the mean (M), Standard Deviation (SD) and percentage of change.

Histological investigations:

For the histological investigations, small parts of the liver from the control and the treated animals were fixed in neutral formalin. After the fixation period, the tissues were washed in saline solution 0.9% NaCl, dehydrated through a graded series of ethyl alcohol, cleared in xylene, embedded in the parablast, sectioned at a thickness 5-6microns, mounted on the glass slides and stained with haematoxylin and eosin for general morphological studies. Sections were studied and the tissues were compared with the control.

RESULTS

Serum Liver Function tests:

AS shown in tables(1,2,3,4,5) all liver function parameters (AST, ALT, ALP, Totlal billirubin) were highly increased in Diazinon group, while treatment with vitamin C was ameliorated these parameters to some extent.

On the other hand, serum total protein and serum albumin were highly significant decreased in Diazinon group but Vitamin C ameliorated it to some extent too (Tables 6, 7).

Histological and histopathological Studies:

1. **Control Groups-**Microscopic examination of sections from the liver of the control rats showed normal histology of the liver, each liver lobe is seen to be made up of hepatic lobules. The lobules are roughly hexagonal and consist of plates of hepatocytes radiating from a central vein. The central vein joins to the hepatic vein to carry blood out from the liver. Between the hepatocyte plates are liver sinusoids as seen in figures, 1, 2, 3 and 4.
2. **Diazinon groups (5 and 10 Weeks)-**Treating animals with Diazinon caused several histopathological alterations in the liver. After 5 weeks of treatment, the central blood vessels were congested and dilated; the blood sinusoids were dilated and lymphocytic infiltrations were detected, also the hepatic portal vein was congested and bile ducts were degenerated (Figs.5&6). The hepatocytes plates were disrupted and the nuclei of the hepatocytes were mostly exhibited pyknotic or karyolitic nuclei. Moreover, macrophages and some necrotic areas were observed (Figs. 7& 8).

Severe histopathological alterations were detected in rat liver examined after 10 weeks of treatment with Diazinon. Complete hepatic cords disarray; large dilated central veins and dilated blood sinusoids were obviously detected (Figs. 9 & 10). Also, invasion of inflammatory cells and necrotic cells were greatly encountered within the hepatic tissue and the blood vessels, central and portal veins were severely congested and bile ducts were degenerated(Figs. 11 & 12).

3-Diazinon + vitamin C Group (D+C 5 weeks): The histology of the liver tissue from Diazinon and vitamin C-treated animals for 5 weeks showed that vitamin C could exert ameliorative effects. Where slightly normal histological structure of the hepatocytes, central veins, blood sinusoids were demarcated (Fig. 13). Meanwhile, less degeneration was found in the hepatic tissue; represented by slightly dilated blood sinusoids and some necrotic cells with normal architecture of the hepatic cords (Fig. 14).

4-Diazinon + vitamin C Group (D+C 10 weeks):

The histology of the liver tissue from Diazinon and vitamin C-treated animals for 10 weeks exhibited slightly normal histological structure of hepatocytes, central vein, normal bile ducts, blood sinusoids, nuclei and also less degenerative changes, dilated blood sinusoids, dilated central blood vessels, congested hepatic portal vein, some pyknotic hepatocyte nuclei and eosinophilic appearance of the hepatic cells as seen in figures (15 &16).

DISCUSSION

Organophosphate insecticides induce biochemical and histopathological changes in several organs, such as liver, kidney, immune system, pancreas and cardiac and vascular walls in animals [10, 11].

The present study was undertaken to investigate the possible protective effect of vitamin C against Diazinon- induced adverse effects on the performance of rats, biochemical indices and liver histopathological alterations following 5 and 10 weeks of Diazinon administration.

The obtained data revealed significant elevation in serum ALT, AST, GGT, ALP and total bilirubin in rats after the treatment with Diazinon. Elevation of transaminases (AST, ALT) was considered to be a more sensitive measure in evaluating liver function and damage [12].In addition to transaminases elevation, ALP increased in most hepatic diseases as well as in both intrahepatic and extrahepatic obstructive diseases [13].

Diazinon was found to increase malondialdehyde level and decrease antioxidant enzymes in rat erythrocytes [14, 15]. The observed hepatotoxicity in the present work may be attributed to the production of free radicals and involvement of oxidative stress generated by Diazinon treatment. **Hatoff**,[16] reported that elevations in serum levels of these enzymes were mostly attributed to acute hepatocellular damage or extrahepatic obstruction, or both. These enzymes were secreted to blood in hepatocellular injury and their levels increased.[3]The liver cells are the sites of toxic action of Diazinon, which affects mitochondrial membrane transportation in liver and caused swelling of mitochondria in hepatocytes resulting in increased of biochemical indices and liver enzymes[17]**.** Also, **Choudhary *et al*.**[18] attributed the elevation in ALT and AST levels in rats to hepatotoxicity causing permeability alterations and leakage of lysosomal enzymes causing enhanced released enzymes.

However, administration of vitamin C along with Diazinon caused slight decrease in AST and ALT levels suggested the ameliorative effects of vitamin C. In accordance; **Carr andFreis**[19]and**Lebas**[20]reported that vitamin C supplementation can help the stressed animals by maintaining the normal metabolic functions of the body.

Results of the present work indicated that Diazinon induced histopathological alterations in the liver of rats. The liver showed congestion of veins, lymphocytic infiltrations, pyknosis and necrotic areas of the hepatocytes. Similarly; **Sarhanand Al-Sahhaf**[21] reported that intoxicated rabbits with Diazinon resulted in blood vessel congestion, leucocytic infiltrations in the liver parenchyma in addition to cytoplasmic vacuolation, fatty degeneration and pyknotic nuclei in the hepatocytes.

El-Shenawy[22] reported that intoxicated mice with Diazinon resulted in hydropic degeneration, necrosis and focal microvesicular steatosis in liver.**Jacqueson**[23] and **Anthony*et al*.**[24] observed that the liver of male wistar rats chronically treated with sublethal doses of Diazinon sustain a form of hepatic injury characterized by cellular lipid accumulation. **Abdel-Salam and Ford**[25]showedthat Diazinon induced liver and kidney damage. Liver seemed to be mostly affected by different doses of the pesticides. The present results are similar to those of **Choudhary *et al*.**[26]who revealed that the treatment of rats with endosulfan, caused liver damage which included dilation of vessels, degenerative changes included binucleated cells, hypertrophy of hepatocytes cytoplasmic vacuolation and lymphocytic infiltration.

Hyperplasia of hepatocytes, necrosis, lymphocytic infiltrations and steatosis were observed in rats treated with 1/20 LD50 of Diazinon[27].However, the liver cells are the sites of toxic action of Diazinon, which affects mitochondrial membrane transportation in liver and caused swelling of mitochondria in hepatocytes resulting in increased of biochemical indices and liver enzymes[28].Cytoplasmic vacuoles develop due to accumulation of ions and water in cytosol and rapidly pass through leaky membranes of cell organelles. Massive

accumulation of fluids in the vacuoles may finally lead to cell lysis[29].

The results of the present study showed that using of vitamin C reduced the hepatic toxicity of the insecticide Diazinon in male albino rats to some extent. **Abbas**[30] reported that oxidative stress-induced tissue damage can be prevented or ameliorated by favoring the balance towards a lower oxidative stress status. Furthermore, it seems that the ameliorative effect of vitamin C involves the maintenance of antioxidant capacity in ameliorating the tissue against oxidative stress.

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Table 1- Serum Aspartate Transaminase (AST) (U/L) in albino rats protected with vitamin C against chronic treatment by Diazinon.

Table	No.	Control	Diazinon	Diazinon + Vit. C
5 Weeks	Mean	63.17	98.33 **	77.33 **
	SD	±5.23	±15.19	±8.12
	% Ch.		55.7 ↑	22.4 ↑
10 Weeks	Mean	68.17	123.00 **	90.33 **
	SD	±5.19	±17.74	±7.84
	%Ch.		80.4 ↑	32.5 ↑

Table 2- Serum Alanine Transaminase (ALT) (U/L) in albino rats protected with vitamin C against chronic treatment by Diazinon.

Table	No.	Control	Diazinon	Diazinon + Vit. C
5 Weeks	Mean	34.67	70.17 **	59.00 **
	SD	±3.39	±4.31	±7.21
	%Ch .		102.4 ↑	70.2 ↑
10 Weeks	Mean	38.33	126.67 **	84.83 **
	SD	±2.16	±19.12	±6.85
	%Ch .		230.5 ↑	121.3 ↑

Table 3- Serum γ-Glutamate Transaminase (GGT) (U/L) in albino rats protected with vitamin C against Diazinon toxicity.

Table	No.	Control	Diazinon	Diazinon + Vit. C
5 Weeks	Mean	6.70	26.17 **	18.83 **
	SD	±0.53	±4.92	±5.91
	%Ch .		290.6 ↑	181.0 ↑
10 Weeks	Mean	7.02	29.67 **	23.33 **
	SD	±0.25	±5.13	±5.01
	%Ch .		322.7 ↑	232.3 ↑

Table 4- Serum Alkaline Phosphatase (ALP) (U/L) in albino rats protected with vitamin C against chronic treatment by Diazinon.

Table	No.	Control	Diazinon	Diazinon + Vit. C
5 Weeks	Mean	38.17	72.33 **	51.17 **
	SD	±3.66	±5.39	±3.87
	%Ch .		89.5 ↑	34..1 ↑
10 Weeks	Mean	69.50	141.17 **	85.67 ns
	SD	±10.67	±25.40	±11.29
	%Ch .		103.1 ↑	23.3 ↑

Table 5- Serum Total Bilirubin (T. Bili.) (mg/dl) in albino rats protected with vitamin C against chronic treatment by Diazinon.

Table	No.	Control	Diazinon	Diazinon + Vit. C
5 Weeks	Mean	0.78	2.55 **	1.82 **
	SD	±0.12	±0.37	±0.37
	%Ch .		226.9 ↑	133.3 ↑
10 Weeks	Mean	0.88	2.78 **	1.98 ***
	SD	±0.15	±0.16	±0.31
	%Ch .		215.9 ↑	125.0 ↑

Table 6- Serum Total Protein (g/dl) in albino rats protected with vitamin C against chronic treatment by Diazinon.

Table	No.	Control	Diazinon	Diazinon + Vit. C
5 Weeks	Mean	6.72	4.50 **	5.08 **
	SD	±0.23	±0.47	±0.79
	%Ch .		-33.0 ↓	-24.4 ↓
10 Weeks	Mean	6.93	4.22 ***	4.80 ns
	SD	±0.24	±0.35	±0.42
	%Ch .		-39.1 ↓	-30.7 ↓

Table 7- Serum Albumin (g/dl) in albino rats protected with vitamin C against chronic treatment by Diazinon.

Table	No.	Control	Diazinon	Diazinon + Vit. C
5 Weeks	Mean	4.10	2.85 ***	3.42 ns
	SD	±0.29	±0.33	±1.11
	%Ch .		-30.5 ↓	-16.6 ↓
10 Weeks	Mean	4.23	2.70 **	3.20 **
	SD	±0.33	±0.42	±0.70
	%Ch .		-36.2 ↓	-24.4 ↓

Where: C = Control, **D** = Diazinon, **D + C** = Diazinon + Vitamin C**, SD** = Standard Division, **% Ch** = Percentage of change from control, **ns**= Non Significant, *****= Significant,******= High Significant.

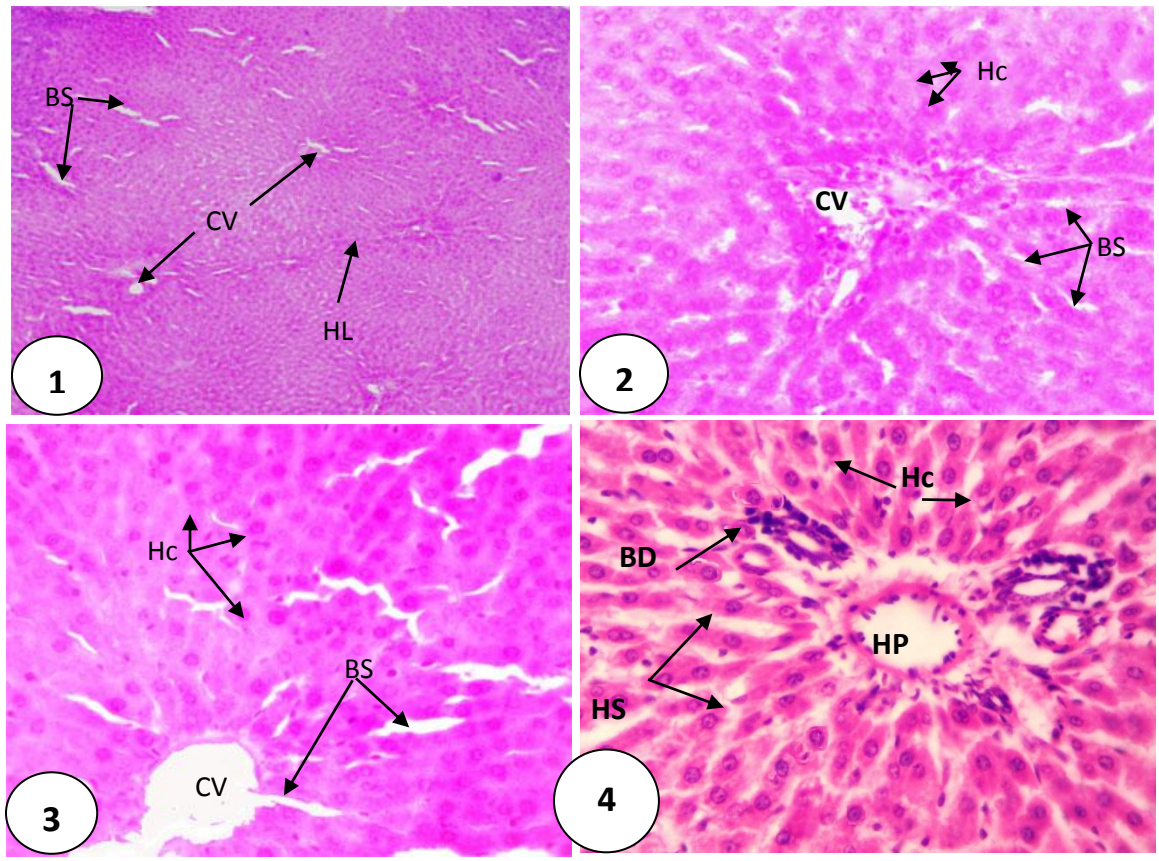

Figure1- Photograph of liver section of control a rat showing normal structures; where: BS: blood sinusoid, CV: central vein and HL: hepatic lobule. (H/E stain X100)

Figure 2-Photograph of liver section of a control rat showing normal structures; where: BS: Blood Sinusoid, CV: central vein and Hc: hepatocytes. (H/E stain X400).

Figure 3- Photograph of liver section of a control rat showing normal structures; where: BS: blood sinusoid, CV: central vein and Hc: hepatocytes. (H/E stain X400).

Figure 4- Photograph of liver section of a control rat showing normal structures; where: BD: bile duct, HP: hepatic portal vein, HS: hepatic strands and Hc: hepatocytes. (H/E stain X400).

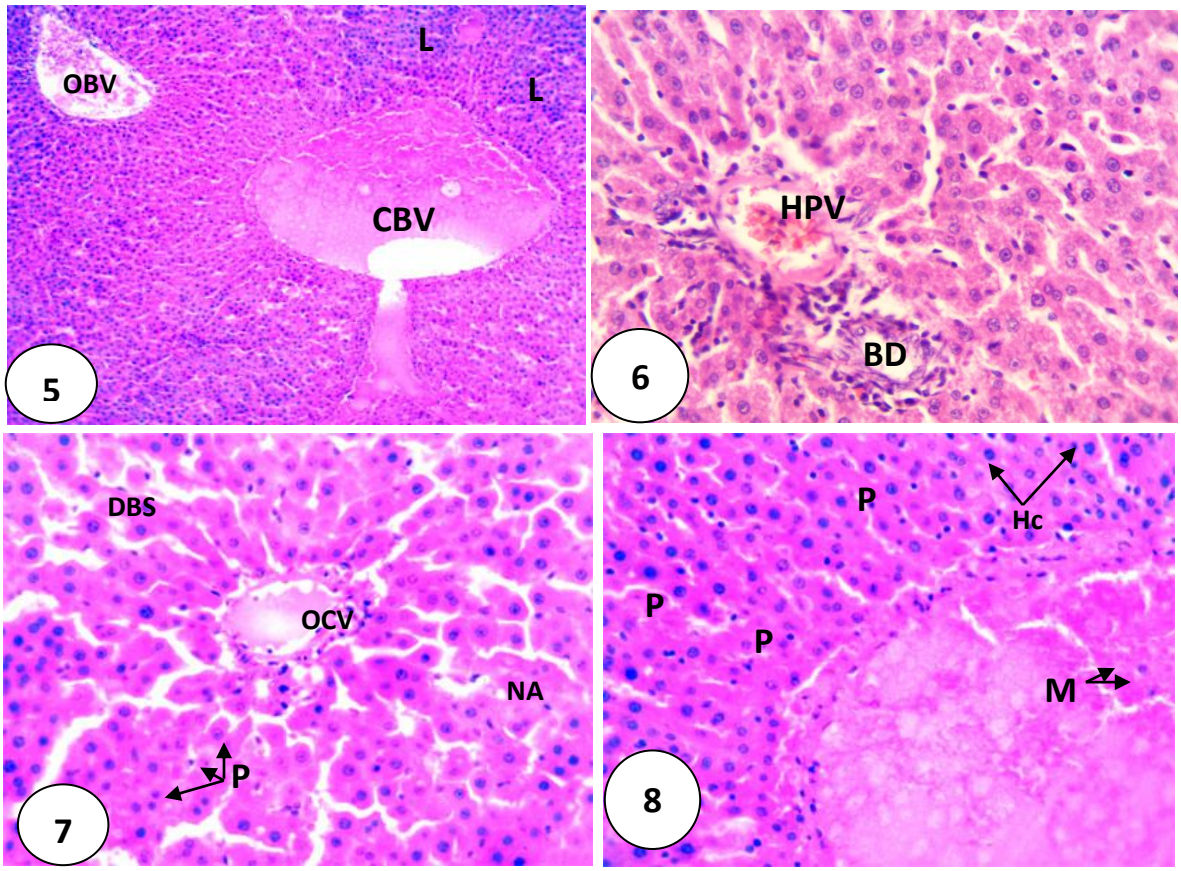

Figure 5- Photograph of liver section of a rat after 5 weeks of treatment with Diazinon showing different histological changes where: CBV: congested blood vessels (Central veins) and L: lymphocytic infiltration. (H/E stain X100).

Figure 6- Photograph of liver section of a rat after 5 weeks of treatment with Diazinon showing different histological changes where: HPV: congested hepatic portal vein and BD: degenerated bile duct (H/E stain X400).

Figure 7- Photograph of liver section of a rat after 5 weeks of treatment with Diazinon showing different histological changes where: CV: congested blood vessel, DBS: dilated blood sinusoids, P: pyknosis and NA: necrotic areas. (H/E stain X400).

Figure 8Photograph of liver section of a rat after 5 weeks of treatment with Diazinon showing different histological changes where: Hc: hepatocytes, P: pyknosis, hemolysed blood cells inside the congested central vein and M: macrophages. (H/E stain X400).

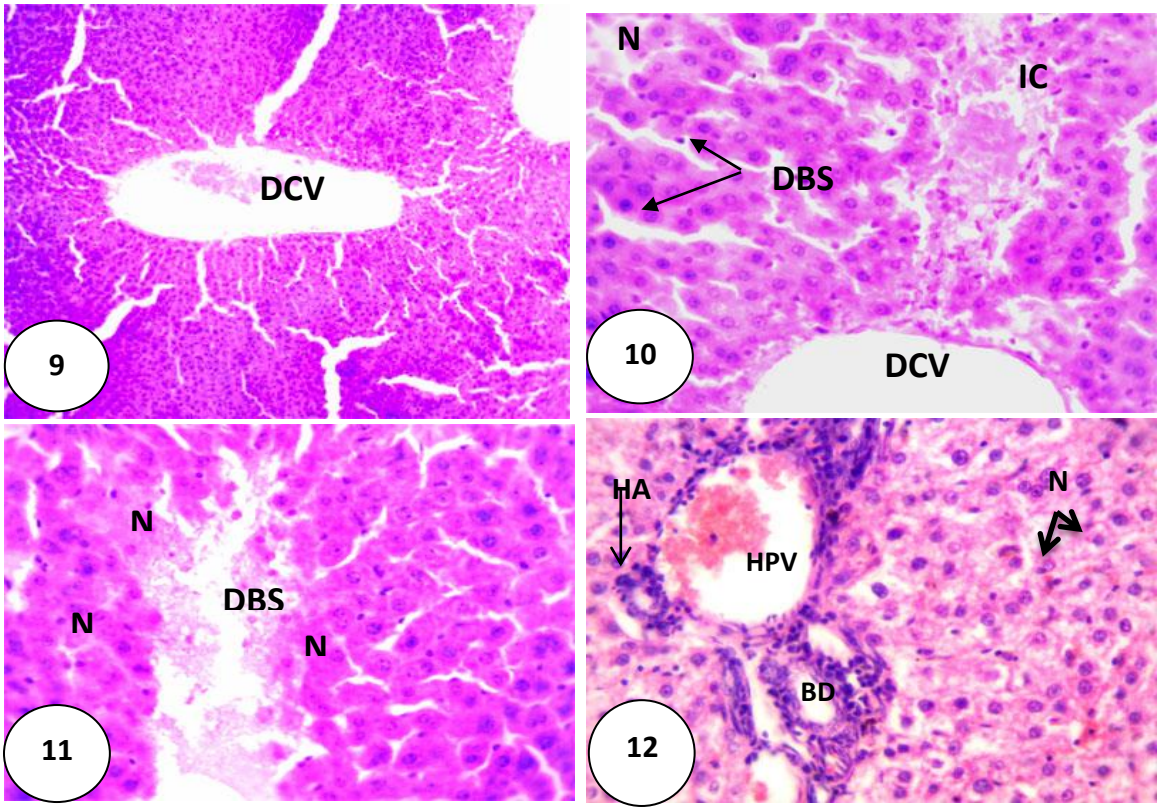

Figure 9 - Photograph of liver section of a rat after 5 weeks of treatment with Diazinon showing DCV: dilated central vein and blood sinusoids, HC: hepatic cords. (H/E stain X100).

Figure 10- Photograph of liver section of a rat after 5 weeks of treatment with Diazinon showing severed histological changes where: DCV: dilated Central vein, DBS: dilated blood sinusoids, N:necrotic areas and IC: Inflammatory cells. (H/E stain X400).

Figure 11- Photograph of liver section of a rat after 5 weeks of treatment with Diazinon showing severed histological changes such as: dilated central vein, DBS: dilated blood sinusoids, IC: inflammatory cells and N: necrosis. (H/E stain X400).

Figure 12- Photograph of liver section of a rat after 5 weeks of treatment with Diazinon showing severed histological changes where: HPV: congested hepatic portal vein, HA: distorted hepatic artery, BD: degenerated bile duct, pyknotic and karyolitic nuclei of hepatocytes and N: necrotic cells (H/E stain X400).

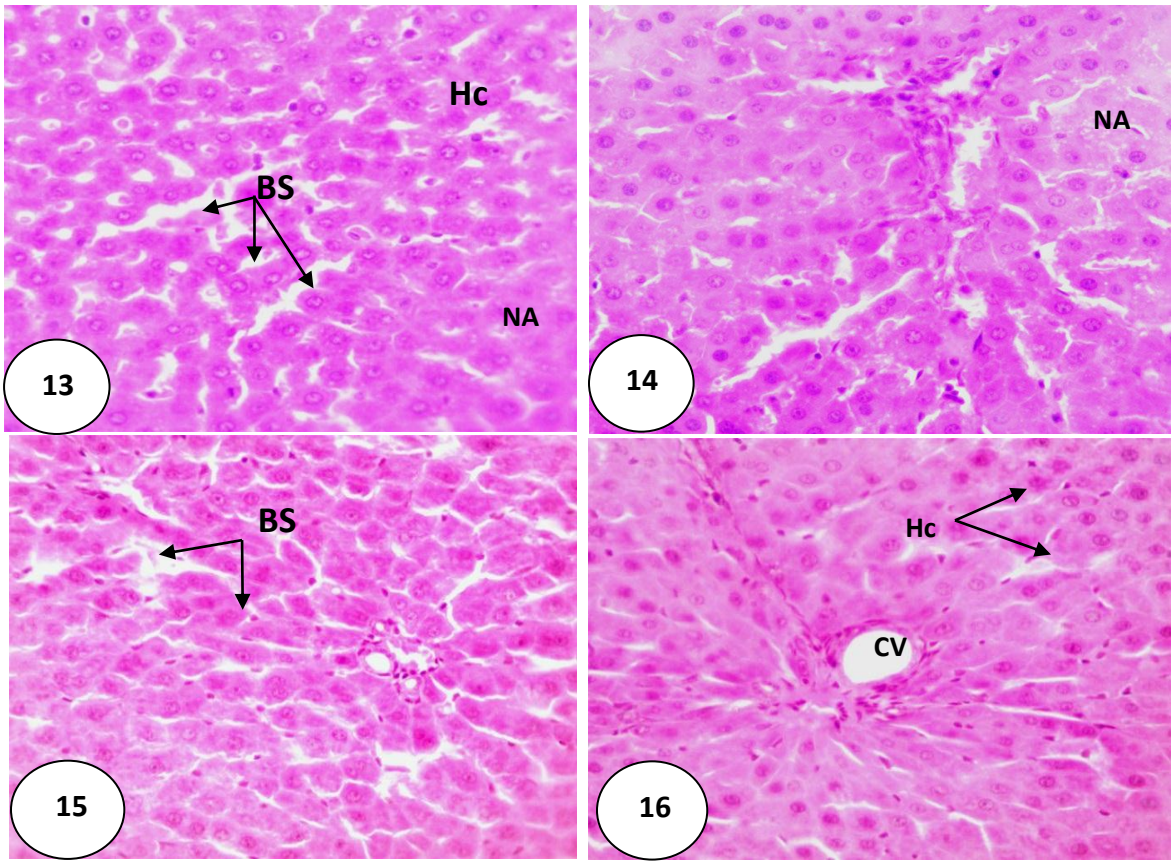

Figure13 -Photograph of liver section of a rat after 5 weeks of treatment with Diazinon and vitamin C showing less degenerative effects where: BS: blood sinusoids, Hc: hepatocytes and NA: necrotic area (H/E stain X400).

Figure 14- Photograph of liver section of a rat after 5 weeks of treatment with Diazinon and vitamin C showing less degenerative effects where: NA: necrotic area. (H/E stainX400).EC

Figure 15- Photograph of liver section of a rat after 5 weeks of treatment with Diazinon and vitamin C showing less degenerative effects where: BS: slightly dilated blood sinusoids.(H/E stain X400).

Figure 16- Photograph of liver section of a rat after 5 weeks of treatment with Diazinon and vitamin C showing less degenerative effects where: CV: slightly dilated central vein and Hc: eosinophilic hepatocytes H/E stain X400).